DETERMINATION OF RUBBER IN FLESHY AND WOODY TISSUE PLANTS

212

BY

C. O. WILLETS, C. L. OGG, W. L. PORTER, AND M. L. SWAIN

DETERMINATION OF RUBBER IN FLESHY AND WOODY TISSUE PLANTS[1]

By C. O. Willits, C. L. Ogg, W. L. Porter, and M. L. Swain (Eastern Regional Research Laboratory,[2] Philadelphia 18, Pa.)

SYNOPSIS

A procedure is presented for the determination of rubber in kok-saghyz roots, cryptostegia leaves, and similar fleshy plant material; and modifications of the Spence-Caldwell method are recommended for the analysis of guayule and other woody plants. Directions are given for the preparation of laboratory samples of these plants, including the grinding procedures, for sampling both in the gross and on a laboratory scale, and for suitable methods of moisture determination.

---

[1] Natural Rubber from Domestic Sources. Paper No. 13, Eastern Regional Research Laboratory.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

INTRODUCTION

Rubber-bearing plant tissues can be divided into two general classes, fleshy and woody. Each requires a different procedure for the determination of rubber, irrespective of whether the rubber exists in cells or in laticiferous ducts.

Two imported, rubber-bearing plants, cryptostegia (a vine native to Madagascar and India) and *Kok-saghyz taraxacum* (the Russian dandelion) are richest in rubber in the fleshy leaves and roots, respectively; whereas the rubber in such plants as guayule, rabbitbrush, and pingue is contained in woody tissue. Cryptostegia has been well established in this country as an ornamental shrub, and experimental plantings have been made for the purpose of studying this plant as a source of rubber. While the available literature on cryptostegia is not extensive, the analytical data of Hall and Long (4) and of Polhamus, Hill, and Elder (7) indicate that a rubber content of from 3 to 5 per cent dry weight basis, may be expected in cryptostegia leaves, depending upon variety and season harvested. A considerably lower percentage (1 per cent) has been reported for the stems. Whittenberger, Brice, and Copley (13) have shown that the rubber in cryptostegia leaves occurs at least partially in a laticiferous duct system paralleling the veinous system of the leaves and extending into the stems. A larger fraction of the rubber exists within the individual leaf cells.

Unlike cryptostegia, kok-saghyz is a recent import to the Western Hemisphere, having been introduced into the United States from Russia. Reported rubber contents of Russian-grown roots range from 5 to 28 per cent in individual specimens, and values as large as 15 per cent on gross lots are apparently common. The rubber occurs in greatest concentration in the root fraction. The Russian literature abounds in "rapid" methods for the determination of the approximate rubber content of kok-saghyz, but few instructions for accurate analysis are given. The "rapid" methods are in general designed to permit an estimation of the approximate rubber content of many individual roots as an aid in plant selection for propagation experiments. These methods are extremely varied in design; those of Stolbin (11) and of Koyalovich (5) depend upon isolation of the crude rubber by removing other plant constituents through successive digestions with sodium hydroxide and sulfuric acid solutions. The weight of the residue, which consists chiefly of impure rubber, is corrected by an empirically determined factor in order to approximate the true rubber content. The method of Goryainov (2) attempts to correlate the electrical conductance of a living root with its rubber content.

Guayule (*Parthenium argentatum* Gray), a shrub native to the Chihuahuan desert of Mexico and the United States, has long been known to be a good source of rubber. Examination of sections of this plant revealed that the largest concentrations of rubber exist in cells within the bark.

Two similar plants, rabbitbrush (*Chrysothamnus nauseosus*) and pingue (*Actinea Richardsoni*) have also been investigated as sources of rubber. Relatively few methods for the analysis of these plants for rubber have been described, and only two have been used to any extent. These are (a) the extraction method of Hall and Goodspeed (3), in which the rubber is estimated by weighing the crude rubber-benzene extract obtained after resins and other non-rubber-benzene solubles have been removed by preliminary acetone extraction; and (b) the method of Spence and Caldwell (10), which has been expressly developed for the determination of rubber in guayule. The latter relies on digestion of the ground tissue of the plant with dilute sulphuric acid followed by autoclaving to effect disintegration of the cell structures so that the resinous components of the plant may be made available to the action of the acetone, and the rubber to the action of the benzene in the subsequent extractions

Since no evaluation of the accuracy and reliability of existing methods for the determination of rubber in cryptostegia and kok-saghyz had previously been made, several of the more promising of these methods have been tested and an improved method has been developed. For the analysis of guayule and the other woody plants, the Spence-Caldwell method has been improved by simplifying the technics and by shortening the time required in the preliminary steps. The purity of the benzene-extracted rubber from each plant has been established by a gravimetric rubber tetrabromide procedure involving the principles of the Edison (1) method.

In addition to the development of satisfactory methods for the quantitative extraction of rubber, it was necessary to devise schemes of sampling and particularly methods of grinding so that satisfactory samples for laboratory analysis could be obtained.

PREPARATION OF SAMPLE

Kok-saghyz.—After the roots have been windrowed, a representative sample of kok-saghyz is taken by either the random-grab handful or the alternate shovel methods. After selection of the sample, the roots are washed to remove the large amounts of adhering soil. Such washing must be carefully done to prevent small cuts and bruises, which lead to "bleeding."

To aid in the subsequent grinding, the roots are then dried until the moisture content has been reduced to approximately 5 per cent. The percentage loss in weight due to this partial drying must be determined if it is desired to convert the percentage of other constituents to the "as received" basis. The drying is conveniently done in a mechanical convection oven at 65°C., and the required drying time, which varies with the size of roots and original moisture content, usually ranges from 12 to 24 hours. The method of grinding kok-saghyz is important, since the rubber in the dried roots exists in a coagulated form within the fibrous walls of the latex system.

The partially dried, embrittled roots of the large representative sample are broken by hand or ground to pass a $\frac{1}{4}$-inch screen in either a Wiley mill or a Ball and Jewell cutter. After the sample of ground or broken roots has been well mixed, it is reduced to a laboratory-size sample by the cone and quarter method, or by some mechanical device such as the Jones riffler or Boerner sampler.

The laboratory sample is then ground to pass a 3-mm. mesh screen in a Raymond hammer mill modified to be similar to the mill described by Ross and Hardesty (9). When dried kok-saghyz roots are ground to the desired fineness in a Wiley mill, particles of rubber form agglomerates which are retained on the screen, and the whole mill becomes gummed with a resinous rubbery plant exudate. By freezing the sample with solid carbon dioxide and keeping it frozen during grinding, most of these faults can be avoided, but the ground product still exhibits less uniformity of rubber distribution and yields lower percentages of rubber on extraction than that prepared in the hammer mill. Table 1 shows the rubber results obtained on various grinds of a sample of kok-saghyz roots ground to various particle sizes. Both the per cent benzene extract and the per cent rubber hydrocarbon as determined by the tetrabromide procedure (14) are given. The low rubber recoveries obtained from the kok-saghyz samples ground in the Wiley mill are due to retention of rubber in the mill and to the relatively large particle size of the sample.

Cryptostegia.—A representative gross sample of cryptostegia leaves is obtained and dried prior to subsampling in a manner similar to that used for kok-saghyz. Contamination of the leaves with sand or other extraneous material is seldom serious, and washing is therefore not usually necessary. It is preferable to grind the gross sample of partially dried, friable leaves in a Wiley mill having a 3-mm. mesh screen rather than in a hammer mill, since too extensive grinding reduces it to an impalpable powder which is difficult to handle in the subsequent extraction. The ground sample is mixed and then reduced to laboratory size by the cone and quarter or other suitable method.

Guayule.—A representative sample of a large lot of the fresh or dried plants is obtained by the random selection of 8 or 10 plants out of every hundred. The entire plants are then chopped in a Ball and Jewell mill to pass a $\frac{1}{2}$-inch screen, and the moisture is reduced to 5 or 6 per cent by drying in a mechanical convection air oven at 65°C. The partially dried sample is then ground in a modified Raymond hammer mill having a 3-mm. mesh screen and reduced to laboratory size by any of the previously mentioned methods.

The fineness and character of the grind are of great importance in the efficiency of the solvent extraction of the rubber and also in the degree of uniformity of the sample obtained. When two-year old guayule, pingue, and rabbitbrush were ground in a Wiley mill having a 3-mm. screen, satisfactory laboratory samples were not obtained unless the plants were frozen with solid carbon dioxide during grinding. More satisfactory samples were prepared with greater ease by using the hammer mill, and in addition solid carbon dioxide was not required. Specimens of higher rubber content, such as 7- to 14-year old guayule, were very difficult to grind in the Wiley mill even when the material was kept frozen with Dry Ice, since large agglomerates of crude rubber and resin separated out during

TABLE 1.—*The influence of grind on the per cent rubber found*

PLANT	MILL	SCREEN SIZE	PER CENT BENZENE EXTRACT M.F.B.*		PER CENT RUBBER HYDROCARBON** M.F.B.*	
		Mm.		*Ave.*		*Ave.*
Kok-saghyz	Wiley	3	4.00 4.39	4.20	3.98 4.30	4.14
	Hammer	7	4.34 4.40	4.37	4.32 4.38	4.35
	Hammer	3	4.77 4.83	4.80	4.76 4.83	4.80
	Hammer	2	4.74 4.83	4.79	4.70 4.77	4.74
	Hammer	1	4.82 4.87	4.85	4.80 4.80	4.80
Guayule-A	Hammer	3	14.54 14.42	14.48	14.37 14.29	14.33
	Wiley	3	15.17 13.48	14.33	14.98 13.02	14.00
Guayule-B	Hammer	3	14.70 14.94	14.82	14.59 14.84	14.72
	Wiley	3	14.39 16.01	15.20	14.02 15.90	14.96

* M.F.B. = Moisture-free basis.
** By tetrabromide method (14).

the grinding process. The uneven distribution of these rubber agglomerates made accurate sampling impossible, and the difficulty of obtaining their complete solution in the extractors led to incomplete extraction. Uniform samples of high rubber specimens were obtained by grinding such plant material in the hammer mill to pass the 3-mm. mesh screen.

The influence of grind on the uniformity of the sample and on the completeness of extraction, as shown by the amounts of rubber extracted, is illustrated in Table 1 for two samples of 14-year old guayule.

The greater uniformity of the hammer-mill ground samples, reflected in the extraction data, was confirmed by microscopic examination of the unextracted samples. The Wiley-mill grind of frozen and unfrozen guayule samples always contained large ill-distributed particles of separated rubber and resin, whereas in the hammer-mill grind the woody particles were fairly evenly coated with the rubber-resin mixtures.

Although the samples of both fleshy and woody tissue were ground to pass the 3-mm. mesh screen in both mills, screen analyses of the two grinds (Table 2) indicate that the hammer-mill ground samples contained a much higher percentage of fine particles than did those ground in the Wiley mill.

TABLE 2.—*Influence of grind on particle size distribution*

SCREEN MESH	PER CENT OF SAMPLE PASSING SCREEN			
	KOK-SAGHYZ		GUAYULE	
	HAMMER MILL	WILEY MILL	HAMMER MILL	WILEY MILL
200	22.1	6.5	0.3	0.0
120	30.6	10.7	2.4	0.1
100	38.4	12.8	4.0	0.3
80	44.4	17.5	8.0	1.5
60	57.0	24.7	17.9	6.4
40	77.3	42.7	45.5	24.6
30	87.4	61.7	73.0	49.5
20	95.3	86.7	96.6	78.3

The decreased particle size may contribute to the increased efficiency of the extraction when the hammer-mill grind is employed, although the improved rubber distribution in the sample is believed to be the most important factor.

MOISTURE DETERMINATION

An accurate moisture determination on the ground plant material is of considerable importance if the rubber contents of various samples are to be expressed on a comparable basis. Before accurate moisture determinations can be made, it is necessary to establish the proper drying period and temperature for each plant. A method of determining the appropriate drying temperature by a series of time-per cent loss isotherms has been fully described by Porter and Willits (8). The correct temperature for moisture determination on cryptostegia and guayule in a mechanical convection oven is 110°C. Constancy of weight for a 10-g. sample of cryptostegia is generally achieved in 30 to 60 minutes, and in approximately 90 minutes for a sample of guayule of the same size.

We found the correct temperature and time for moisture determination on kok-saghyz in a mechanical convection oven to be 100°C. for 30 minutes. The lower temperature and shorter time for drying the kok-saghyz samples may be attributed to their high sugar content. The values obtained by drying at this time and temperature are in close agreement with moisture values established by vacuum drying.

RUBBER AND RESIN EXTRACTION

The rubber content of plants has generally been determined by the extraction methods of Hall and Goodspeed, or of Spence and Caldwell. Various extraction apparatus and periods of extraction have been used with both methods. In this laboratory a continuous drip extractor of the Smalley type (Fig. 1), modified to hold a 30-×80-mm. thimble and equipped with ground glass joints at both the condenser and flask connections, has been used for all extractions. This extractor gives a hot extraction with a rapid exchange of solvent, and is preferred to both the Soxhlet and Bailey-Walker types. The thimble is a heavy-walled glass test tube with a round, perforated bottom. In use, the perforations are covered by a plug of glass wool or cotton, which serves to retain the sample. The diameter of the extraction thimble is such that the thimble may be fitted into a Walter crucible holder, permitting the use of suction to free the sample of solvent retained at the end of the water and acetone extractions. The extraction flasks used for both the acetone and benzene are 125-ml. Pyrex No. 4100, and those for the water extraction of guayule are 250 ml.

Tests conducted with these extractors indicate that under ideal conditions the acetone extraction approaches completion in from 3 to 6 hours. Nevertheless, an acetone extraction period of 16 hours is used in practice in order to avoid discrepancies introduced by differences in rate of drip and rate of percolation. Similarly, while a 16-hour extraction period suffices for the extraction of rubber from most samples, the time is usually extended to 24 hours to insure against variations between individual extractors and samples.

A 4-hour drying period at 65°C. in a mechanical convection oven is adequate for achieving complete dryness of the acetone extracts, provided that the larger share of acetone is first removed by evaporation on the steam bath. The syrupy rubber solutions obtained on evaporation of the

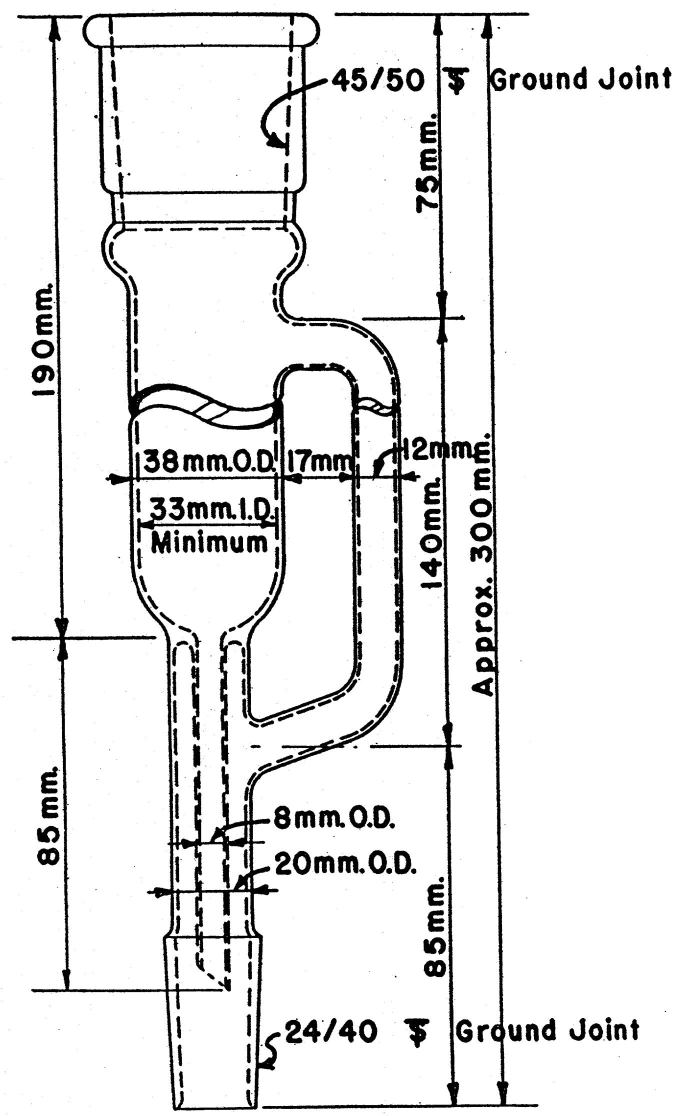

FIG. 1.—Extraction Tube, Smalley (special)

benzene extract on the steam bath are dried to constant weight at 50°C. in a vacuum oven under 5 mm. pressure. A 3-hour drying period under these conditions has been adequate for the rubber films from all samples so far encountered. Oxidation of the rubber film does not take place to any detectable degree under these conditions of short-time drying under high vacuum. Phenyl-β-naphthylamine, diphenylparaphenyl diamine, or di-β-naphthyl-para-phenylene added to the benzene before the extraction process, or to the benzene extract before evaporation and drying of the rubber, had no effect on the values for rubber obtained. Antioxidants are therefore not used in the regular procedure.

Kok-saghyz.—Test runs on ground kok-saghyz roots, using the Hall and Goodspeed method, yielded rubber extracts seriously contaminated by pigments and other plant constituents. Increase in time of the acetone extraction did not lead to any improvement in the purity of the benzene extracts obtained, and it became evident that some preliminary treatment other than acetone extraction was required. Samples were therefore extracted according to the procedure of Spence and Caldwell, since it was considered possible that the boiling of the sample in dilute sulfuric acid and the autoclaving included in the preliminary steps of this method, might hydrolyze and dissolve the benzene-soluble, non-rubber constituents in the benzene extracts obtained in the previous simple acetone-benzene extraction. Although pure rubber extracts were obtained by the Spence-Caldwell method, incomplete extractions and erratic values resulted.

During the acetone extraction of some samples of kok-saghyz after the Spence-Caldwell preliminary treatment, a white, gelatinous, pectin-like material was deposited in the thimble and extractor. Analysis on roots confirmed the presence of as much as 17 per cent pectin. The erratic low rubber values obtained might have been due to the encasement of the rubber-bearing particles by a gelatinous pectic substance which was impervious to the benzene solvent. A preliminary treatment is therefore desirable which will (a) remove non-rubber, acetone-insoluble plant constituents that are benzene-soluble, and (b) either remove the pectin or prevent its gelatinization.

Extraction with an oxalic acid solution at a pH of 2.5 under reflux provides an excellent means of removing the pectin from apples (6). Kok-saghyz pectin is similarly soluble in oxalic acid solutions of the same pH. One-half hour refluxing of the dried, ground roots in this medium led to nearly complete removal of the pectin. However, this reflux method did not lend itself to the analysis of large numbers of samples without considerable investment in equipment and space; therefore a 2-hour digestion of the sample in oxalic acid at 80°–100°C. was substituted. This treatment removes so much of the pectin that the amount remaining in the roots is without effect in the subsequent extractions, provided the sample is distributed between layers of cotton and washed well with water before and after introduction into the thimble. If the oxalic acid-treated sample is not dispersed in cotton, incomplete extraction of the rubber results, as shown by microscopic examination of these benzene-extracted residues.

Satisfactory rubber extractions can be obtained if the sample, after being digested in dilute sulfuric acid and autoclaved as in the Spence-Caldwell procedure, is removed from the thimble, distributed between layers of cotton as in the previous method, reinserted in the thimble, and washed with water prior to the acetone extraction. The sample cannot be dispersed on the cotton previous to the acid and autoclaving treatments, since the action of the strong acid disintegrates the cotton. Lamb's wool, although somewhat more acid resistant than cotton, is partially disintegrated during the autoclaving, and glass wool is not sufficiently retentive to hold the finer particles.

To study the effect of the pretreatments, samples were analyzed by the Hall-Goodspeed, Spence-Caldwell, and oxalic acid methods. In each method the samples were distributed between layers of cotton prior to the acetone extractions to avoid differences in extraction arising from variations in packing of the sample in the thimbles. These variations occur if the regular procedures are followed, since the different pretreatments alter the character of the material. The results are presented in Table 3. Both the benzene extract, and the rubber hydrocarbon values obtained by the tetrabromide procedure, are given; and the closeness of their agreement may be taken as a measure of the purity of the benzene extracts.

As indicated by the figures in Table 3, extraction of kok-saghyz samples which have been given the Spence-Caldwell preliminary treatment yields results similar to those obtained when the oxalic acid preliminary treatment is used, provided that the treated samples are dispersed on cotton prior to extraction. Even distribution of the soaked and partially digested sample over the surface of the cotton mat is both difficult and time consuming. Moreover, a total of nine hours is required to complete the Spence-Caldwell preliminary treatment, whereas that for the oxalic acid requires only two hours. The latter method is therefore distinctly preferable because of its ease of manipulation and economy of time. Owing to the extraction of non-rubber material, the rubber values (per cent benzene extract) obtained when the sample is not pretreated, as in the Hall and Goodspeed procedure, are high.

The rubber films obtained by drying the benzene extract of kok-saghyz roots which have been given a preliminary oxalic acid treatment have been found by combustion methods to contain carbon and hydrogen totaling 98.8 to 99.4 per cent. Such films are essentially free of ash, nitrogen, and sulfur. The carbon and hydrogen ratio corresponds to an average empirical formula of $C_5H_{8.04}$, which is in satisfactory agreement with that of C_5H_8 for rubber.

The oxalic acid method is applicable to the analysis of kok-saghyz roots, crowns and leaves, as well as the residues encountered in the milling process of such fractions. The rubber content of the kok-saghyz roots varies over a considerable range, depending upon maturity, locality grown, season harvested, and strain. The average rubber content of the portion of the plant usually harvested, which consists of the roots and crown plus one inch of the leaves, approximates 5 per cent of the dry weight of the

Table 3.—*Rubber values obtained by extraction of samples of Kok-saghyz and cryptostegia with the Hall-Goodspeed, Spence-Caldwell, and oxalic acid pre-treatments*

Sample	Hall and Goodspeed[1]				Spence and Caldwell[1]				Oxalic acid			
	Benzene extract M.F.B.[3]		Rubber hydrocarbon[2] M.F.B.[3]		Benzene extract M.F.B.[3]		Rubber hydrocarbon[2] M.F.B.[3]		Benzene extract M.F.B.[3]		Rubber hydrocarbon[2] M.F.B.[3]	
	Per cent	*Ave.*	*Per cent*	*Ave.*	*Per cent*	*Ave.*	*Per cent*	*Ave.*	*Per cent*	*Ave.*	*Per cent*	*Ave.*
Kok-saghyz	4.53		4.23		4.00		4.00		4.23		4.23	
RK 1422	4.67		4.19		4.04		4.04		4.21		4.21	
		4.60		4.21		4.02		4.02		4.22		4.22
Kok-saghyz	3.94		3.71		3.67		3.67		3.81		3.79	
RK 1402	4.29		3.86		3.70		3.70		3.76		3.76	
		4.12		3.79		3.69		3.69		3.76		3.78
Kok-saghyz	5.33		4.77		4.75				4.54		4.50	
RKX_1	5.21		4.54		4.77		4.67		4.69		4.56	
		5.27		4.66		4.76				4.62		4.53
Cryptostegia	3.08		2.48		5.72		5.41		5.43		5.30	
RC 2100	4.24		2.60		5.63		5.41		5.44		5.35	
		3.66		2.54		5.68		5.41		5.44		5.33
Cryptostegia	3.16		2.51		3.88		3.44		3.85		3.80	
RC 1796	3.37		2.53		3.89		3.63		3.87			
		3.27		2.52		3.89		3.54		3.86		
Cryptostegia	3.37		2.57		4.84		4.12		4.46		4.39	
RC 1484	3.50		2.54		4.99		4.15		4.50			
		3.44		2.55		4.92		4.14		4.48		

[1] Kok-saghyz samples were dispersed on cotton just prior to extractions.
[2] By tetrabromide method (14).
[3] M.F.B. = Moisture-free basis.

plant. The rubber content of kok-saghyz leaves may approach 2 per cent but usually does not exceed 1 per cent. The distribution of rubber, resins and dry weight among root, crown and leaf fractions is shown in Table 4. These results, obtained on a sample of spring-harvested roots grown in Florida, are typical of the distribution figures generally found.

Cryptostegia.—Previously reported determinations of the rubber content of cryptostegia leaves have been made by the Hall and Goodspeed method, either in its original or in a slightly modified form. Test runs on cryptostegia in which this simple acetone-benzene extraction procedure was used inevitably resulted in benzene extracts highly contaminated with plant pigments and other plant constituents, whereas the extracted plant residue contained considerable amounts of unextracted rubber as revealed by microscopic examination (12). Use of the Spence-Caldwell method also

TABLE 4.—*Distribution of rubber, resins, and dry weight among root, crown, and leaf fraction of some Kok-saghyz plants*

FRACTION	PER CENT BENZENE EXTRACT M.F.B.*	PER CENT ACETONE EXTRACT M.F.B.*	PER CENT TOTAL RUBBER OF PLANT	PER CENT TOTAL RESINS OF PLANT	PER CENT TOTAL DRY WEIGHT OF PLANT
Roots	8.0	3.7	72.3	42.3	49.7
Crowns	4.3	4.7	18.6	25.8	23.9
Leaves	1.9	5.3	9.2	31.9	26.4
Total Plant	5.5	4.4			

* M.F.B. = Moisture-free basis.

resulted in highly colored, contaminated benzene extracts although complete extraction of the rubber was generally secured. With cryptostegia, the Spence-Caldwell method frequently produced deposition of a gelatinous mass over the lower surfaces of the thimbles, particularly in the stems of the extractors. This suggested that the materials impeding the action of the solvents might be pectin, as in the case of the kok-saghyz, and that a satisfactory extraction could be secured only if such materials were removed from the samples prior to extraction. The presence of pectin in cryptostegia leaves was later confirmed. The same oxalic acid preliminary treatment used for kok-saghyz was consequently introduced in the analysis of cryptostegia and gave greatly improved results. The two-hour heating period at a temperature of 80–100°C. in oxalic acid solution adjusted to a *p*H of 2.5 sufficiently removes the pectin. Following its removal both resins and rubber may be extracted by the customary solvents. The benzene extracts are translucent, owing to traces of pigments which give them a slight greenish-yellow color. From their absorption bands these pigments have been identified as pheophytin and carotene. Since the impurities producing the visible coloration do not amount to more than 0.02 per cent of the dry sample, the error in rubber determination is negligible.

The results of the analyses of cryptostegia leaves by the Spence-Caldwell, the Hall and Goodspeed, and the oxalic acid methods, are given in Table 3. They illustrate the relatively close agreement between the rubber hydrocarbon values by the tetrabromide method and the benzene extract values when the preliminary oxalic acid treatment is used. The more drastic conditions of mineral acid digestion and autoclaving used in the Spence-Caldwell preliminary treatment disintegrate the leaves so completely that excessive contamination of the benzene extract with non-rubber constituents occurs, and erroneously high rubber recovery figures result.

Some evidence indicates that a small fraction of cryptostegia rubber is acetone-soluble, and is therefore lost whenever a preliminary acetone extraction is used. Bromination of acetone-extracted material from both cryptostegia leaves and isolated leaf rubber yields a product which is white, amorphous, and alcohol- and acetone-insoluble, and which corresponds in bromine content to rubber "tetrabromide." In all samples the amount of this acetone-soluble "rubber" was from 0.2 to 0.4 per cent of the dry weight of the leaves. Since some doubt may exist as to the propriety of considering this brominated material from the acetone extract as true rubber, the loss of this fraction is disregarded.

Carbon and hydrogen determinations on rubber films resulting from the evaporation and vacuum drying of cryptostegia benzene extracts, obtained following an oxalic acid preliminary treatment, correspond to an empirical formula ranging from $C_5H_{8.01}$ to $C_5H_{8.05}$. Slow evaporation and drying of the benzene solution of cryptostegia rubber in air, and in contact with platinum, resulted in a film totaling only 90 per cent for carbon and hydrogen, whereas the total was more than 99 per cent after rapid vacuum drying. This oxidation is apparently the result of the extreme thinness of the film produced on the walls of the platinum combustion boat, since it has not been encountered during the usual evaporation and drying of cryptostegia benzene extracts in the extraction flasks. It does indicate, however, that a fraction of cryptostegia rubber is readily oxidizable, and therefore oxidation might occur during the extraction of the rubber by the solvent process. Consequently, extractions were carried out (a) in the usual manner, in which no special precautions were taken to prevent oxidation, (b) with antioxidants, and (c) in an atmosphere of nitrogen. Variations in the amounts of extracted rubber, with or without these precautions to prevent oxidation, were less than 1 per cent of the total rubber.

The oxalic acid method has been used on both the leaves and stems of the cryptostegia plant, and the rubber content of mature leaves on a dry-weight basis has been found to be approximately 5 per cent for the Florida hybrid, 4 per cent for the Mexican hybrid, and 3 to 4 per cent for the grandiflora species. Cryptostegia stems from these sources generally contain a maximum of 1.0 per cent rubber.

Guayule.—The original Spence-Caldwell preliminary treatment consisted of boiling the sample for three hours in 1 per cent sulfuric acid, autoclaving for three hours at 15 pounds gage pressure, and leaching with water for three hours. A series of tests on the various types of woody plant material indicated that this preliminary treatment was unnecessarily long and that each of the three steps could be shortened by an hour without effect on the final result. Thus, two hours of boiling in 2 per cent sulfuric acid followed by two hours of autoclaving at 15 pounds proved to be as effective in disintegrating interfering plant structures as the longer treatment in the Spence-Caldwell procedure. The three-hour leach with water at 60°C. has been replaced by a two-hour extraction with water in the Smalley extractors. This continuous hot water extraction not only is as effective in removing water-soluble material as the method recommended by Spence and Caldwell, but it also requires less attention.

A comparison of the results obtained with the Spence-Caldwell method and those obtained by the modified method on a variety of materials is given in Table 5.

TABLE 5.—*Results obtained by the original and by the modified Spence-Caldwell methods of rubber extraction*

SAMPLE	SPENCE-CALDWELL METHOD		MODIFIED METHOD	
	BENZENE EXTRACT	RUBBER HYDROCARBON*	BENZENE EXTRACT	RUBBER HYDROCARBON*
	Per cent	*Per cent*	*Per cent*	*Per cent*
Guayule (14 year)	14.80	14.61	14.90	14.72
Guayule (2 year)	6.73	6.51	6.74	6.51
Pingue	0.96	0.89	1.00	0.89
Rabbitbrush	0.51	0.47	0.53	0.48

* By tetrabromide method (14).

As the slight differences in the results obtained with the two methods do not exceed the allowable deviation between duplicates, they may be regarded as insignificant. If the difference between per cent benzene extract and per cent rubber hydrocarbon as determined by the tetrabromide procedure, is taken as a criterion of the relative degree of contamination of the extracts, the two methods again give essentially the same result. The extracts obtained by either method are seldom completely pure. The dried benzene extract usually has a slight brown or green tinge, which is most pronounced in the extracts obtained from young guayule. Rubber films prepared from benzene extracts, obtained in the course of the analysis of young or old guayule shrub, have given carbon and hydrogen values corresponding to empirical formulas ranging from $C_5H_{7.98}$ to $C_5H_{8.17}$. The essential purity of the rubber films is also shown by the general close agreement between the direct weight of rubber as determined by weight of the benzene extract, and the rubber hydrocarbon as determined by the gravimetric tetrabromide procedure.

Completeness of extraction of the rubber has been checked on a large number of samples by microscopic examination of the extracted plant residues. Residues from low rubber samples, on such examination, are essentially free of rubber. Even after all precautions in grinding, sampling, and preliminary treatment have been observed, residues from samples of 7- to 14-year old guayule containing 15 to 16 per cent rubber always show a trace of unextracted rubber in spite of variations in sample size, solvent, type of extractor, and with or without the addition of the solvent aid (trichloroacetic acid).* The constancy of the amount of rubber extracted from high rubber guayule under varying conditions of extraction suggests that the small percentage of rubber remaining in the extracted residue may exist in some highly insoluble or atypical form.

The acetone solubles and benzene extract have been determined by the modified Spence-Caldwell method on guayules shrubs of various ages, grown under varying cultural conditions, and on rabbitbrush and pingue. Both the latter plants were consistently low in rubber, ranging in concentrations from 0.60 to less than 2 per cent, and having an average rubber content of approximately 1 per cent of the dry weight of the plant. Specimens of two-year old guayule ranged in rubber content from 2 to 7 per cent on a dry weight basis, depending somewhat on variety and growing conditions. Seven- to fourteen-year old guayule contained from 13 to 17 per cent rubber, the 15 per cent level being seldom exceeded.

The percentage distribution of rubber and resins in various parts of the different plants is of practical as well as scientific interest. Accurate knowledge of the concentration of rubber in various portions of the plant has potential value in determining which parts may be economically discarded. The rubber and resins contents have been determined by the modified method in the various parts of both young and old guayule plants and in a five-year old rabbitbrush plant. The results are given in Table 6.

ANALYTICAL PROCEDURES

Kok-saghyz and Cryptostegia

Select representative gross samples of kok-saghyz and cryptostegia and prepare the laboratory sample for analysis by cleaning, partial drying, grinding, and subsampling, as described previously. (After the laboratory sample has been bottled, it should never be remixed, since this causes the rubber particles to float to the top of the sample.)

Take a 10-gram sample for moisture and dry it in a mechanical convection oven, at 110°C. to constant weight ($\frac{1}{2}$ to 1 hour) for cryptostegia, and at 100°C. for $\frac{1}{2}$ hour for kok-saghyz.

For the resin and rubber determinations, weigh out 2.5 grams of the laboratory sample of the kok-saghyz roots, or 5 grams of the laboratory sample of the crypto-

* The use of trichloroacetic acid was suggested by Mr. H. Boucher of the Intercontinental Rubber Company.

Table 6.—*Distribution of rubber and resins in guayule and rabbitbrush*

Fraction	Per cent acetone soluble M.F.B.*	Per cent benzene extract M.F.B.*	Per cent of total acetone solubles in plant	Per cent of total benzene extract in plant	Per cent of total dry weight of plant
Guayule (1 year, 4 months old)					
Root	4.9	3.8	17.2	23.1	19.1
Lower stem	4.6	4.4	6.9	12.2	8.1
Branches	5.3	5.3	28.2	46.8	28.4
Leafy portion	5.8	1.3	47.7	17.9	44.4
Guayule (8 years old)					
Root	9.9	9.0	5.8	3.4	5.8
Lower stem	8.8	8.0	6.2	3.7	7.0
Branches	9.9	18.0	70.0	83.2	69.8
Leafy portion	10.2	8.4	18.0	9.7	17.4
Rabbitbrush (5 years old)					
Root	2.2	1.1			
Lower stems	3.4	1.6			
Upper twigs	11.7	0.6			
Outer bark	5.2	2.9			
Inner bark	2.4	2.7			
Wood	4.2	0.9			

* M. F. B. = Moisture-free basis.

stegia leaves. Transfer the sample to a 250-ml. beaker, add 100 ml. of 0.8% oxalic acid solution (pH 2.5), and heat for two hours at 80 to 100°C. Transfer the digested sample with the aid of hot water, onto an acetone-washed absorbent cotton mat approximately $4 \times 6 \times \frac{1}{4}$ inches, supported by a wire screen. Distribute the sample evenly over the surface of the mat to within one inch of each edge, wash with 100–200 ml. hot water and then fold the side edges of the cotton in to form a rectangle of $2\frac{1}{2} \times 6$ inches. Roll the mat into a cylinder $2\frac{1}{2}$ inches long and approximately one inch in diameter, and insert in the glass thimble with a minimum of twisting. Place the thimble in a Walter crucible holder supported in a suction flask, and wash with 400–500 ml. of hot water, using gentle suction. Remove the excess water by increasing the suction, then transfer the thimble to the modified Smalley extraction tube (Fig. 1). Extract for 15–20 hours with 75 ml. acetone contained in a tared 125-ml. extraction flask. At the end of the extraction remove the flask, evaporate to near dryness on a steam bath, dry to constant weight at 65°C. (approximately four hours in a mechanical convection oven), cool in a desiccator and weigh.

Before making the benzene extraction remove the acetone from the thimble by again using the Walter crucible holder assembly and gentle suction. Return the acetone-free thimble to an extractor which has been washed and oven dried. Extract for 24 hours with 75 ml. of benzene contained in another tared 125-ml. extraction flask. After the extraction is complete, evaporate the benzene extract to near dryness on a steam bath, dry to constant weight in a vacuum oven at 50°C. (approximately 3 hours at less than 5 mm.), cool in a desiccator and weigh. To obtain the values on an "as received basis" it is necessary to determine the percent loss in weight of the sample during the 65°C. drying, as well as the moisture in the laboratory sample.

Guayule

Obtain a representative sample of guayule shrubs and grind, dry, and reduce it to laboratory size as described under Preparation of Sample. Determine moisture on a 10-gram sample by drying at 100°C. in a mechanical convection oven to constant weight (approximately 90 minutes).

For the resin and rubber determination, transfer a 5-gram sample to a glass extraction thimble in which the perforations have been covered with a plug of pyrex fibrous glass. Insert a second plug above the sample to prevent loss during the subsequent operations. Immerse the thimble in a 2% sulfuric acid solution until the upper level of the sample is beneath the surface of the dilute acid, and boil for two hours. At the end of the boiling period transfer the undrained thimble to an autoclave, and steam for two hours at 15 pounds gage pressure. Place the thimble in a modified Smalley extractor, connect with a 250-ml. extraction flask containing approximately 175 ml. of water and extract for two hours. Place the thimble in a Walter crucible holder and partially dry the sample by aspiration. Replace the water flask by a tared 125-ml. extraction flask containing 75 ml. of acetone and extract the sample for 18 hours. At the end of this period, again dry the sample by aspiration and return the acetone-free sample to a clean, dry extractor. Attach a second tared 125-ml. extraction flask containing 75 ml. benzene, and extract for 24 hours. Obtain the dry weights of the acetone and benzene extracts as in the previous procedure, and calculate the percentages of each. This method is also applicable to the determination of rubber in rabbitbrush and pingue.

ACKNOWLEDGMENT

Acknowledgment is made to the Forest Service and to the Bureau of Plant Industry, Soils, and Agricultural Engineering, of the U. S. Department of Agriculture, and to the Société Hatiano-Americaine de Developpement Agricole, for supplying the plants for these studies.

The authors are indebted to R. T. Whittenberger for his microscopic examination of the extracted plant tissues for residual rubber.

LITERATURE CITED

(1) Edison, Thomas A., Unpublished laboratory notes.

(2) Goryainov, M. N., "Method of Determining the Rubber Content in the Living Roots of Rubber Bearing Plants." *Vsesoiuzn. Akad. Selsk.-Khoz. Nauk im. V.I. Lenina. Dok.* No. 11, 5–6 (1941).

(3) Hall, H. M., and Goodspeed, T. H., "A Rubber Plant Survey of Western North America," 7, Nos. 6–8; 159–278 (1919).

(4) Hall, H. M., and Long, F. L., "Rubber Content of North American Plants." Publication 313, Carnegie Institute of Washington (1921).

(5) Koyalovich, N. B., "Determination of Rubber in Rubber-Plant Roots." *Caoutchouc and Rubber* (*U.S.S.R.*), 7, 26–9 (1939).

(6) Norris, F. W., and Schryver, S. B., "Pectin Substances of Plants. III. The Nature of Pectinogen and Its Relation to Pectic Acid." *Biochem. J.*, 19, 676–693 (1925).

(7) Polhamus, L. G., Hill, H. H., and Elder, J. A., "The Rubber Content of Two Species of Cryptostegia and of an Interspecific Hybrid in Florida." U.S.D.A. Technical Bulletin 457 (1934).

(8) PORTER, W. L., AND WILLITS, C. O., Moisture in Potato Starch. *This Journal.* 27, 179–194 (1944).

(9) ROSS, W. H., AND HARDESTY, J. O., "Grinding of Fertilizer Samples for Analysis." *Ibid.*, 25, 238–246 (1942).

(10) SPENCE, D., AND CALDWELL, M. L., "Determination of Rubber in Rubber-Bearing Plants," *Ind. Eng. Chem., Anal. Ed.*, 5, 371–375 (1933).

(11) STOLBIN, P., "Simplified Methods of Estimation of the Proportion of Rubber in Kok-saghyz for Purposes of Selection." *Sovetskii Kauchuk*, 2, 19–21 (1935).

(12) WHITTENBERGER, R. T., "Oil Blue NA as a Stain for Rubber in Sectioned or Ground Plant Tissues." *Stain Technology*, 19, 93–8 (1944).

(13) WHITTENBERGER, R. T., BRICE, B. A., AND COPLEY, M. J., "Distribution of Rubber in Cryptostegia as a Factor in Its Recovery," *India Rubber World*, 112, 319–323 (1945).

(14) WILLITS, C. O., SWAIN, M. L., AND OGG, C. L., "The Determination of Rubber Hydrocarbon by a Gravimetric Rubber Tetrabromide Method." Paper to be published.